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13. ABSTRACT ***(Maximum 200 Words)***

Overexpression of the *HER-2/neu* oncogene is common in many types of cancer, including breast cancer, and correlates well with poor overall survival rates in cancer patients. Thus, effective methods for controlling the expression of *HER2/neu* could prove clinically useful. Previous experimental data have indicated that binding of the PEA3 protein to a 6-bp-long sequence on the *HER-2/neu* promoter down-regulates the activity of the promoter and subsequently and preferentially inhibits the growth of *HER-2/neu*-overexpressing cancer cells.
Our research was aimed at developing small molecules able to bind 6-bp-long DNA sequences with high affinity and selectivity in the hopes that such molecules would bind to a promoter region of *HER-2/neu* and selectively inhibit the growth of *HER-2/neu*-overexpressing cells. Using a modular approach that we developed previously, we devised a novel chemical strategy and new building block that allow for greater flexibility in designing and preparing structurally diverse DNA binding agents. This versatile building block, a 3-amino-4-thio-L-lyxo-hexopyranose, was designed to localized in the minor groove and to selectively react with more building blocks and linkers than ever before. We demonstrated its usefulness by designing and synthesizing DNA binding agents aimed at covering 6-bp-long sequences.

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TRANSCRIPTIONAL CONTROL OF BREAST CANCER: MOLECULAR LEGOS AND COMBINATORIAL LIBRARIES

Introduction

The continuing goal in my laboratory at The University of Texas M. D. Anderson Cancer Center has been to create technology that will allow us to develop small-molecule drugs that can control vital cellular processes at the genetic level. We previously developed an innovative modular approach to create high-affinity, sequence-selective DNA-binding agents. Our approach combined molecular modeling and a structure-based rational design with a combinatorial chemistry to create libraries of DNA-binding agents.

Overexpression of the *HER-2/neu* oncogene is common in many types of cancer, including breast cancer, and correlates well with poor overall survival rates in cancer patients. Thus, effective methods for controlling the expression of *HER2/neu* could prove clinically useful. Previous experimental data have indicated that binding of the PEA3 protein to a 6-bp-long sequence on the *HER-2/neu* promoter down-regulates the activity of the promoter and subsequently and preferentially inhibits the growth of *HER-2/neu*-overexpressing cancer cells.

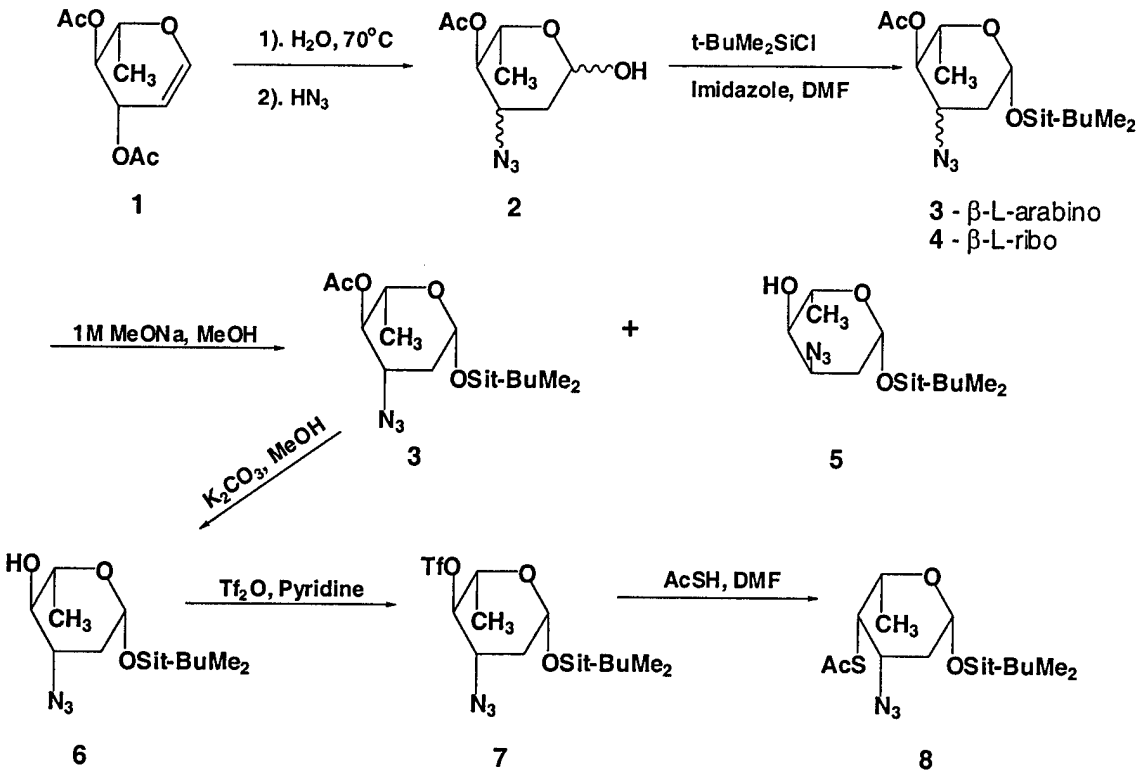
The primary goal of the research supported by this grant was to develop small molecules that could bind to DNA with high affinity and selectivity in the hopes that they would bind to the promoter region and selectively inhibit the growth of *HER-2/neu*-overexpressing cells. Using our modular "Lego" approach, we developed a novel chemical strategy that allows for greater flexibility in designing and preparing structurally diverse DNA binding agents. This versatile

building block, a 3-amino-4-thio-L-lyxo-hexopyranose, was designed to localize in the minor groove and to selectively react with more building blocks and linkers than ever before.

Body

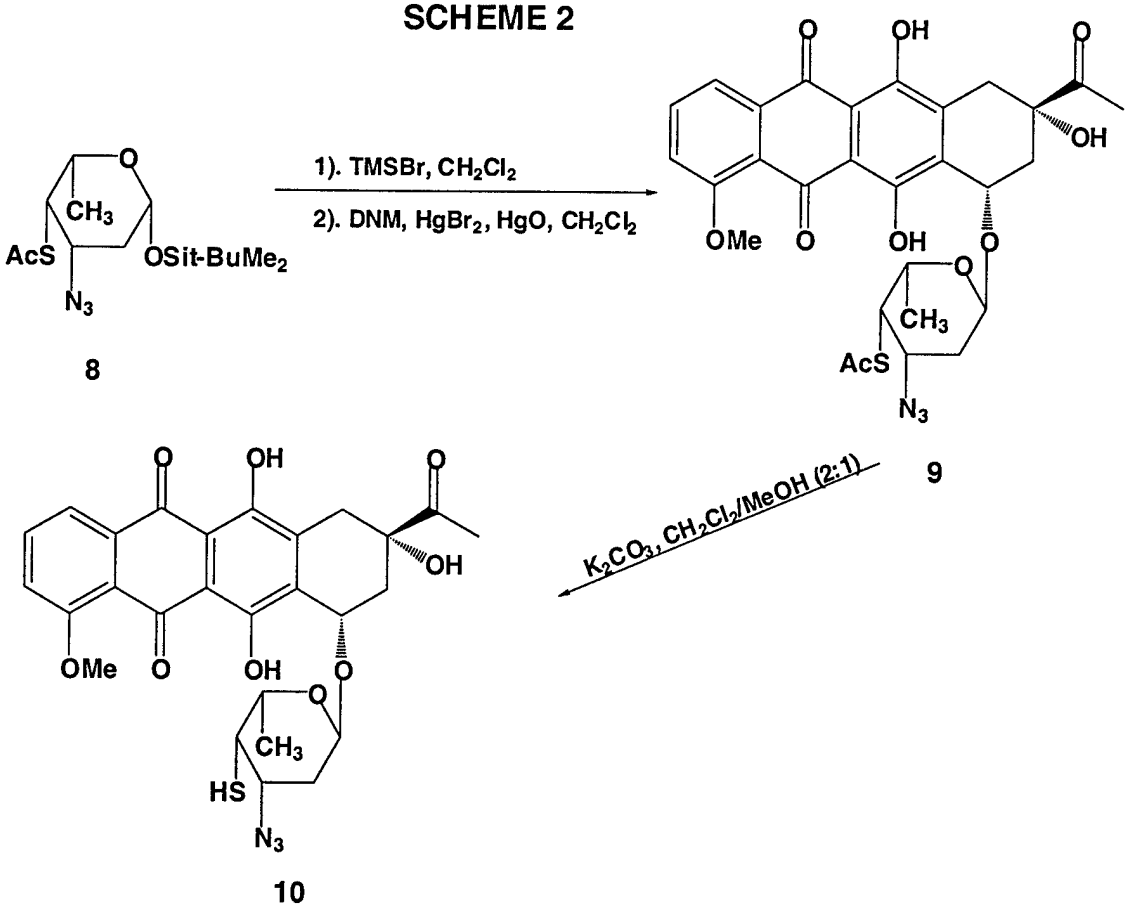
During the one-year grant period, we concentrated on preparing suitable building blocks to create libraries of DNA binding agents that covered at least 6-bp-long binding sequences of DNA. As a result of our efforts, we have designed and synthesized four novel sugars containing amine and thiol groups. Especially useful has been the highly functionalized aminothio hexopyranose 8 with an L-lyxo configuration (Scheme 1) identical to that of natural DNA binding products like daunorubicin.

SCHEME 1



The presence of a thiol group allowed us to use a wider range of cross-linking agents than previously possible because the reactions were very selective and could be carried out in high yield in the presence of other functional groups. We explored alkylation of a thiol and demonstrated that, as expected, it could be performed under milder and more selective conditions than alkylation of an amine. We intentionally preserved the amino group in the sugar moiety. The presence of an amino group was important to ensure the water solubility of the tested compounds but also to further increase the DNA affinity of the final products.

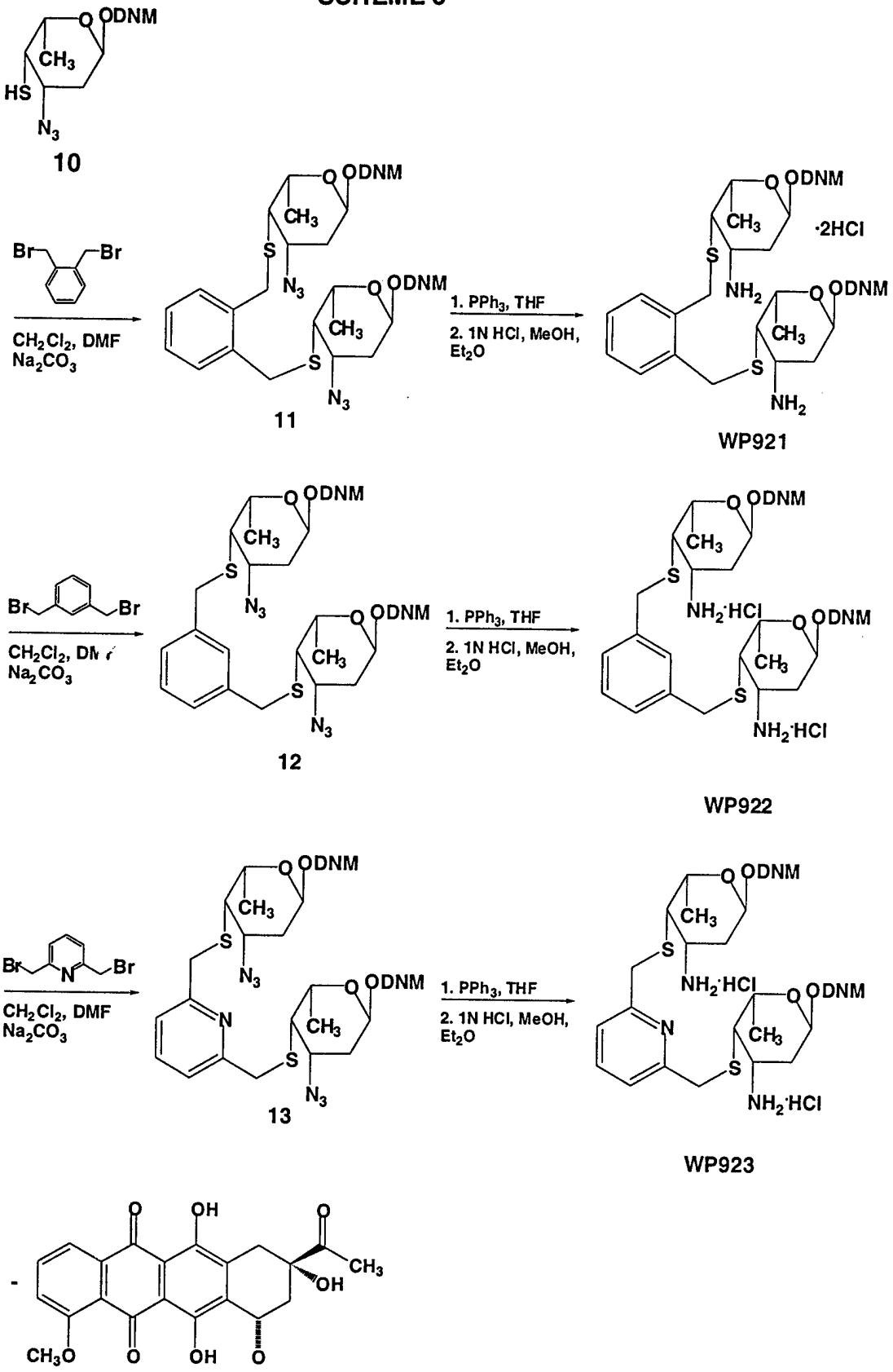
SCHEME 2



Aminothiosugar **8** was coupled with daunomycinone, an intercalating moiety of daunorubicin, to give 4'-S-acetyl-3'-azido-daunorubicin **9**. The subsequent deacetylation yielded 3'-azido-4'-thio-daunorubicin **10** (Scheme 2). 3'-Azido-4'-thio-daunorubicin **10** was used as a 3-bp-binding module and was coupled using different linkers to form DNA binding agents WP909, WP916, WP921, WP922, and WP923 that would cover 6-bp-long sequences and bind with high affinity to DNA (Schemes 3 and 4). Detail description of the synthesis will follow.

The initial evaluation of the cytotoxic potential revealed that compounds WP909, WP916, WP921, WP922, and WP923 displayed differential cytotoxicity ranging from 73 nM to 1.96 μM. Our one-year grant, despite initial synthetic problems, allowed us to solve these problems and to progress to the next step, that is, to develop the methodology essential to preparing a library of the desired compounds. Usefulness of the methodology was demonstrated by synthesizing a new DNA binding agents designed to recognize 6-bp-long sequences. Later, we plan to expand this library of DNA binding agents, compare their suppression of the growth of cancer cells overexpressing HER-2/neu with that of basal HER-2/neu-expressing cell lines, and analyze in detail the DNA binding properties of most selective compounds.

SCHEME 3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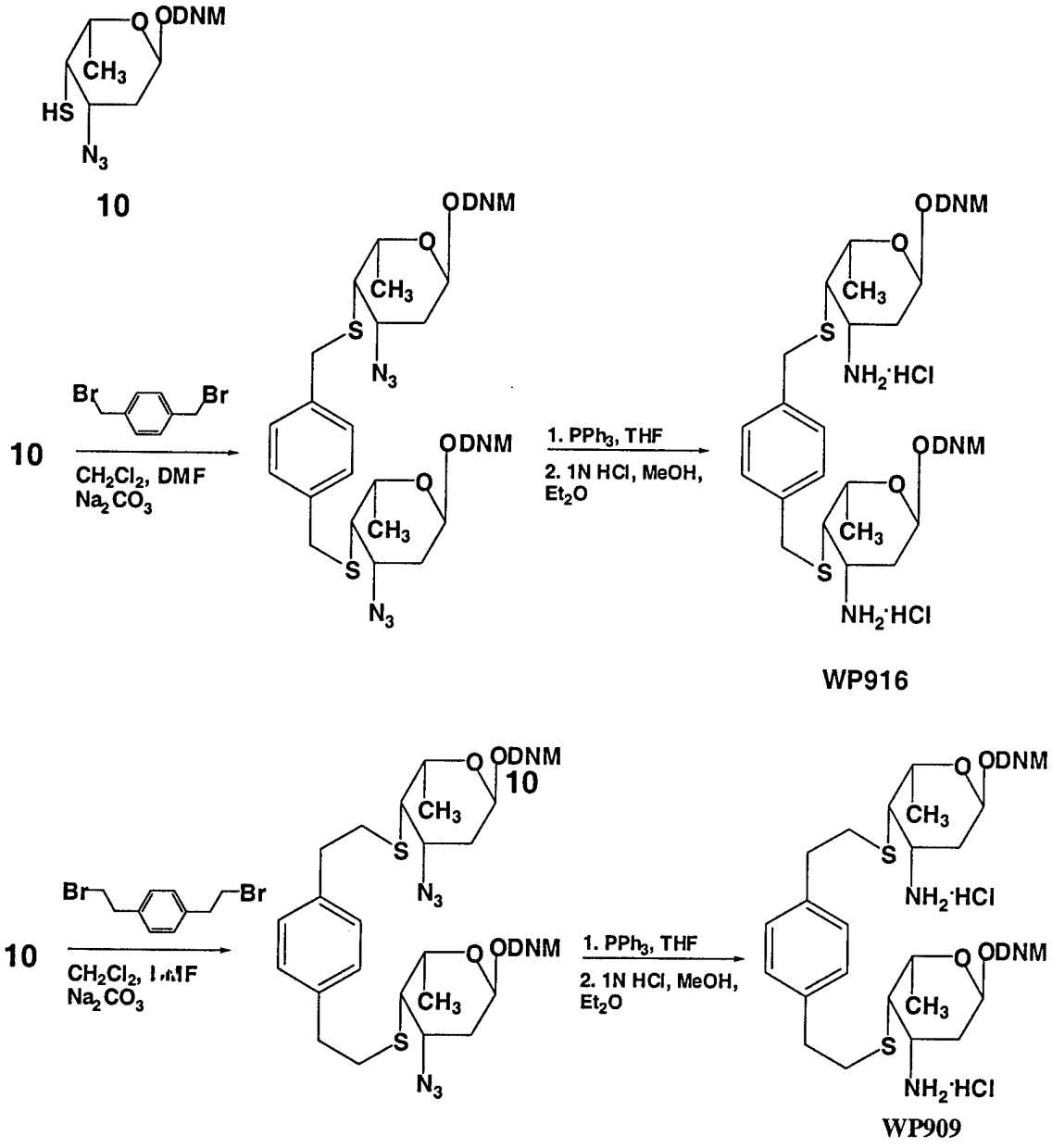
HS
N3
10
Br
Br
CH2Cl2, DMF
Na2CO3
S
11
1. PPh3, THF
2. 1N HCl, MeOH,
Et2O
·2HCl
NH2
WP921
CH2Cl2, DMF
12
NH2·HCl
WP922
13
WP923
DNM -
OH
CH3O

SCHEME 4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CH3
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N3
10
Br
Br
CH2Cl2, DMF
Na2CO3
S
1. PPh3, THF
2. 1N HCl, MeOH,
Et2O
NH2·HCl
WP916
CH2Cl2, DMF
WP909

Desc iption of the synthesis

Synthesis of 4-S-acetyl-3-azido-1-O-tert-butyldimethylsilyl-2,3,4,6-tetradeoxy-β-L-lyxopyranose (8)

1. Hydrolysis of 3,4-di-O-acetyl-L-rhamnal and Michael type addition of HN_3

A mixture of 3,4-di-O-acetyl-L-rhamnal (**1**) (18.43 g, 0.086 mol) and water (740 mL) was stirred at 70°C. Progress of the reaction was monitored by thin-layer chromatography (TLC), and upon completion, the reaction mixture was cooled to 0°C. Sodium azide (12.5 g, 0.192 mol) was added, followed by acetic acid (7.4 mL, 0.13 mol).The reaction mixture was stirred at 0°C for 1 hr and then at room temperature for 12 hr. The reaction mixture was diluted with dichloromethane (500 mL), and the organic and water layers were separated. The water layer was extracted three more times with 200 mL of dichloromethane. The combined organic solutions were washed with a 10% potassium carbonate water solution, then again with water until neutral, and then again with brine. After drying with sodium sulfate, the solvents were removed under reduced pressure, and crude product **2** was used in the next step.

2. Synthesis of 1-O-silylated hexopyroses **3** and **4**

A crude mixture of product **2** was dissolved in dimethylformamide (DMF, 40 mL). Imidazole (28.5 g, 0.38 mol) and t-butyldimethylsilyl chloride (21 g, 0.14 mol) were added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into water (500 mL), and the solution was extracted with hexanes (5x250 mL). The combined hexane extracts were dried

with anhydrous sodium sulfate. The drying agent was filtered, and the solvent was evaporated to dryness to give a yellow oily mixture of **3** and **4.** This mixture was used in the next step without further purification.

3. Selective deacetylation of β-L-ribo isomer **4** to **5** in the presence of **3**

The crude mixture of b-L-arabino and ribo azides **3** and **4** was dissolved in methyl alcohol (300 mL). The solution was cooled to 0°C, and a 1 M solution of sodium methanolate in methanol (2 mL) was added. The reaction mixture was stirred at 0°C for 3 hr (monitored by TLC), and then 1 M HCl (2 mL) was added to neutralize the sodium methanolate. The solution was diluted with water (300 mL) and extracted with hexanes (3x300 mL). The combined extracts were washed with water and then dried. After the drying agent and solvents were removed, the products were separated using column chromatography (Silicagel 60, Merck), with hexane and hexane/ethyl acetate 98:2, 95:5, 9:1 as the eluents, to give 11.4 g (40.3%) of 3-azido-4-O-acetyl-1-O-tert-butyldimethylsilyl-2,3,6-trideoxy-β-L-arabino-hexopyranoside **5.**

4. Deacetylation of β-L-arabino izomer **3**

4-O-acetyl-3-azido-1-O-tert-butyldimethylsilyl-2,3,6-trideoxy-β-L-arabinopyranoside **3** (10 g, 30.3 mmol) was dissolved in methyl alcohol (200 mL). Potassium carbonate (10 g, 72.5 mmol) was added, and the reaction mixture was stirred at room temperature. After the reaction was completed (monitored by TLC), solid inorganic salts were filtered off, and the filtrate was diluted with water

(200 mL) and extracted with hexanes (3x100 mL). The combined extracts were washed with water and dried over anhydrous sodium sulfate. The drying agent was filtered off, and the solvent was evaporated to give pure 3-azido-1-O-(dimethyltertbutyl)silyl-2,3,6-trideoxy-β-L-arabino-hexopyranoside **6** (8.04 g, yield 92.4%).

5. Synthesis of 3-azido-4-S-acetyl-1-O-tert-butyldimethylsilyl-2,3,4,6-tetradeoxy-β-L-arabinopyranoside **8**

A solution of 3-azido-1-O-tert-butyldimethylsilyl-2,3,6-trideoxy-β-L-arabinopyranoside **8** (1.45 g, 5 mmol) and pyridine (3.5 mL, 44.3 mmol) in dichloromethane (50 mL) was prepared and cooled to -40°C. A solution of trifluoromethanesulfonic anhydride (3.5 mL, 20 mmol) in dichloromethane (10 mL) was added dropwise at -40°C. The mixture was stirred for 1 hr at -40°C and then for 1 hr at room temperature. The mixture was diluted with dichloromethane (200 mL) and washed with a 10% water solution of sodium acetate (5x100 mL). The organic solution was dried, and the drying agent was removed. The mixture was evaporated to dryness under diminished pressure. The residue was dissolved with DMF (10 mL). Potassium thioacetate (0.9 g, 7.5 mmol) was added, and the mixture was stirred at room temperature for 0.5 hr (monitored by TLC). The mixture was diluted with ethyl acetate (100 mL), washed with water, and then dried over anhydrous sodium sulfate. The crude product was purified using column chromatography (Silicagel 60, Merck), with hexanes and

hexane/ethyl acetate 98:2 as the eluents, to give 1.6 g (yield 92.6%) of 3-azido-4-S-acetyl-1-O-tert-butyldimethylsilyl-2,3,4,6-tetradeoxy-β-L-arabinopyranoside **8.**

Synthesis of DNA binding agents

1. Synthesis of 4'-S-acetyl-3'-azido-daunorubicin **9**

3-Azido-4-S-acetyl-1-O-tert-butyldimethylsilyl-2,3,4,6-tetradeoxy-β-L-arabino-hexopyranoside 8 (1.52 g, 4.4 mmol) was dissolved in dichloromethane (15 mL). Trimethylsilyl bromide (0.86 mL, 6.6 mmol) was added, and the reaction mixture was stirred at room temperature until the substrate was completely converted into glycosyl bromide, as judged by TLC. The solvents were then evaporated to dryness and the residue dissolved in hexanes and again evaporated to dryness. The process of adding and evaporating the hexanes was repeated three times to obtain a crude glycosyl bromide that was immediately dissolved in dichloromethane (5 mL). This solution was added in five portions to a previously prepared suspension of daunomycinone (0.8 g, 2 mmol), HgO (3.2 g), and $HgBr_2$ (0.8 g) and 4Å molecular sieves in dichloromethane (100 mL). After the reaction was completed, the solids were removed by filtration. The filtrate was washed with a 10% water solution of KI (30 mL) and then with water (3x50 mL). It was dried, and pure compound **9** (2.55 g, yield 95%) was obtained after column chromatography (Silicagel 60, Merck), with dichloromethane as the eluent.

2. Synthesis of 3'-azido-4'-thio-daunorubicin **10**

The 4'-S-acetyl-3'-azido-daunorubicin 9 (2.5 g, 4.09 mmol) was dissolved in 100 mL of a mixture of dimethyl chloride:methanol (2:1, v/v). Potassium carbonate (5 g, 36 mmol) was added, and the mixture was stirred at room temperature. After the reaction was completed, the inorganic solid salts were filtered off, and the filtrate was washed with water until neutral and then dried over anhydrous sodium sulfate. The drying agent and solvent were removed, and the product was purified using column chromatography (Silicagel 60, Merck), with dichloromethane as the eluent, to give 1.98 g (yield 85%) of pure 3'-azido-4'-thio-daunorubicin 10.

3. Synthesis of DNA binding agent WP922

3'-Azido-4'-thio-daunorubicin **10** (1 mmol) and α,α-dibromo-*m*-xylene (0.5 mmol) were dissolved in dichloromethane (5 mL) and DMF (5 mL). Na_2CO_3 (3 mmol) was added, and the mixture was stirred at room temperature for 24 hr. Progress of the reaction was monitored by TLC. After the reaction was completed, the reaction mixture was diluted with dichloromethane (100 mL) and washed with water until neutral. The organic solution was dried over anhydrous sodium sulfate. The drying agent was filtered off, and the solvent was evaporated. The crude product was purified using column chromatography (Silicagel 60, Merck), with dichloromethane, dichloromethane/acetone 98:2 as the eluents.
The resulting 3'-azido-derivative **12** (0.5 mmol) was dissolved in tetrahydrofuran (THF, 10 mL). Triphenyl phosphine (2 mmol) and then water (1 mmol) were added, and the mixture was stirred overnight at room temperature. After the

reaction was completed, the mixture was diluted with THF (20 mL), and 1 N solution of ammonia in methanol was added. The mixture was stirred for an additional 0.5 hr. It was then evaporated to dryness, and the crude residue was placed on the top of the column (Silicagel 60, Merck). The product was eluted with chloroform, chloroform:methanol 98:2, 95:5. Pure, free amine of WP922 was dissolved in methanol, and it was treated with 1 N HCl/methanol. The WP922 was precipitated with diethyl ether, washed with diethyl ether until neutral, and dried under reduced pressure. The structure of the final product was confirmed by nuclear magnetic resonance and elemental analysis.

4. Synthesis of DNA binding agents WP909, WP916, WP921, and WP923

The typical procedure presented above for WP922 was also used to synthesize WP909, WP916, WP921, and WP923. The complete structure of WP916 is shown below in Fig. 1.

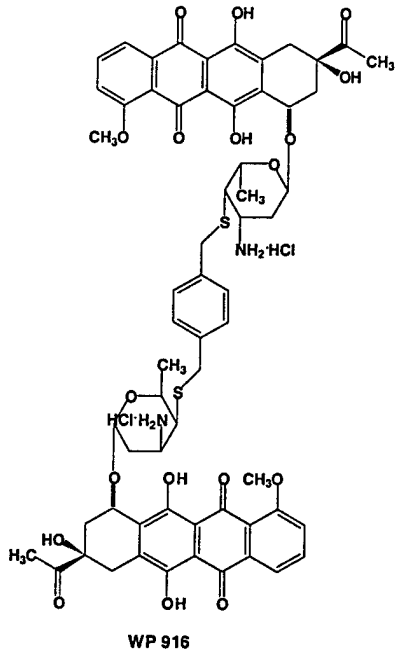

Figure 1. Structure of bisintercalating minor groove binding compound WP916

Key Research Accomplishments

- Design and synthesis of a key building block, 4-S-acetyl-3-azido-1-O-tert-butyldimethylsilyl-2,3,4,6-tetradeoxy-β-L-lyxopyranose
- Preparation of a versatile 3-bp binding module that allows for the preparation of diverse 6-bp-long DNA binding agents
- Synthesis of five new DNA binding agents designed to bind 6-bp-long sequences and the initial assessment of their cytotoxic potential

Reportable Outcomes

An abstract for the National American Chemical Society Meeting (March 23,2003) in New Orleans will be submitted. One publication describing our results is expected. Results of our research will be used to apply for a grant to continue our studies.

Conclusions

We demonstrated the usefulness of our methodology in the preparation of a library of novel compounds with high affinity for DNA able to recognize long sequences of DNA. This methodology allows the creation of a significant number of compounds with the potential to selectively suppress the growth of cancer cells overexpressing *HER-2/neu*.

References N/A

Appendices N/A